Breast-milk vitamin A as an indicator of the vitamin A status of women and infants

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This article reviews the evidence for using breast-milk vitamin A as an indicator of vitamin A status and provides technical information for researchers who want to use this indicator to assess the vitamin A status of women and breast-fed children. Breast-milk vitamin A is a unique indicator for assessing the vitamin A status of lactating women and their breast-fed infants, and has recently been recommended by WHO for use in monitoring global elimination of vitamin A deficiency. Assessing breast-milk vitamin A is less invasive than alternative approaches for assessing a mother's vitamin A status and not at all invasive for her infant. Collection of milk samples in the field is generally feasible and acceptable. Breast-milk vitamin A appears to be an especially good indicator for measuring the impact of vitamin A interventions on women and infants, and for this purpose, it is more responsive than other indicators.

For at least three decades vitamin A deficiency has been recognized to be a public health problem in many parts of the world. Xerophthalmia, the clinical deficiency syndrome that affects the eye and which is caused by vitamin A deficiency, occurs most frequently in young children. The age at which xerophthalmia is likely to occur is influenced by the age of weaning. Xerophthalmia tends to affect a younger age group if the duration of breast-feeding is short, but occurs among 3–4-year-olds in societies where children are breast-fed for several years (*1, 2*). Because children aged 1–5 years are at highest risk of clinical vitamin A deficiency, efforts to assess vitamin A deficiency in populations and interventions to combat vitamin A deficiency have focused on this age group.

Recently, it has been recognized that the consequences of vitamin A deficiency for children include a greater risk of mortality, with both subclinical and clinical vitamin A deficiency beginning at least by 6 months of age (*3–8*). In populations where the prevalence of clinical vitamin A deficiency is of public health importance, improving the vitamin A status of preschool-age children could decrease their risk of mortality by around 23% (range, according to study population, 0-54%) (*9*). Currently available evidence

suggests that this effect on mortality is mediated primarily through a decrease in the severity of common infectious diseases (*10, 11*); the influence of vitamin A on disease incidence has been reported (*10*) but not firmly established (*11, 12*).

Because an excess mortality risk is associated with both clinical and subclinical vitamin A deficiency, and because the latter affects many more people than clinical vitamin A deficiency, assessment of the deficiency and interventions has to be guided by the epidemiology of the clinical *and* subclinical deficiency,[a] rather than only by the epidemiology of xerophthalmia.

This has important implications for assessing the public health importance of vitamin A deficiency in communities. First, the indicators used to assess the public health importance of this condition should reflect the prevalence of *all* vitamin A deficiency, not only clinical deficiency. Thus, commonly used indicator criteria to evaluate the public health importance of vitamin A deficiency in preschool-aged children (such as the prevalence of serum retinol values below a selected cut-off) need to be modified. Second, subgroups of the population in addition to the preschool-age children are of interest in this respect. For example, women of reproductive age and breast-fed infants are two subgroups whose vitamin A status has seldom been assessed in population surveys because xerophthalmia is usually rare among them.

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[a] Here, we use vitamin A deficiency to mean body stores of vitamin A that are not sufficient to maintain optimal concentrations in target tissues. This is generally observed when the body reserves, as determined by the concentration of vitamin A, fall below 20 µg/g liver (*13*).

Subclinical vitamin A deficiency may, however, be common among such groups. Because of the high risk of mortality in infancy, the public health importance of vitamin A deficiency as a contributing factor may be substantial (14). Women who are vitamin A deficient may have infants who suffer from vitamin A deficiency, and the condition may also have negative consequences on their own health (15, 16). Assessment of subclinical vitamin A deficiency in women and infants will require different indicator criteria, or new indicators. The ideal indicator would be acceptable to those being surveyed, simple to determine, and permit estimation of the prevalence of vitamin A deficiency in a community using a reasonable sample size.

WHO has therefore recently recommended that breast-milk vitamin A concentrations be used together with other indicators to assess the prevalence of vitamin A deficiency and to monitor the impact of intervention programmes.[b] The present article discusses the evidence for using breast-milk vitamin A concentration as a potential indicator of vitamin A status in lactating women and breast-fed children, and provides technical information for those who may want to use breast-milk vitamin A levels to assess vitamin A status in these target groups.

Vitamin A partitioning during pregnancy and lactation

Vitamin A is transferred in two ways from mother to offspring: via the placenta during gestation and via the mammary gland (breast milk) during lactation. Of these routes, transfer during lactation is quantitatively more important. Normal birth-weight U.S. infants have median liver vitamin A concentrations of 0.038 µmol/g (range, 0–0.34 µmol/g) (17). On average, a 3.2-kg newborn in the U.S. has stores of 5 µmol vitamin A (assuming that the liver represents 4% of body weight). In contrast, during the first 6 months of life, the breast-fed infant consumes approximately 310 µmol of vitamin A from mother's milk (2.3 µmol/l × 0.75 l/day × 180 days) (18). Thus, normally 60 times more vitamin A is transferred from mother to infant during 6 months of lactation

than is accumulated by the fetus during 9 months of gestation.

The fetus requires vitamin A for normal development, but has no protection against the toxic effects of excess amounts. The placenta is well adapted for such an eventuality and, except in cases of unusually high maternal intakes, allows only the small amount of vitamin A required by the fetus to pass from mother to fetus. Thus, infants, even those of well-nourished mothers, are born with small reserves of vitamin A (15).

Human milk is equally well adapted to protecting the vulnerable neonate from vitamin A deficiency. Colostrum is particularly rich in vitamin A, containing approximately 7 µmol/l (19), and is thus an excellent dietary source of the vitamin during the infant's first days of life. The mature milk of well-nourished women contains around 2.3 µmol/l vitamin A (18), ample to meet the infant's metabolic requirements and to accumulate safe and adequate stores of the vitamin. In addition, vitamin A in human milk is uniquely well absorbed, in part because of the presence of a lipase in the milk that helps the infant to digest the vitamin (20).

The vitamin A content of human milk is, however, dependent on the mother's vitamin A status. In parts of the world where vitamin A deficiency is common, mature human milk typically contains around 1 µmol/l vitamin A (range, 0.4–1.8 µmol/l) (15, 21). It should be emphasized that even when the vitamin A content of mother's milk is low, it is the best dietary source of the vitamin for 0–6-month-olds, and very probably continues to be the best source during the complementary feeding period in later infancy. In addition, breast milk protects the infant against infectious diseases, which may deplete vitamin A stores and can precipitate xerophthalmia if the stores are very low. Thus, breast-feeding provides significant protection against xerophthalmia (22, 23). However, a milk vitamin A concentration of 1 µmol/l is only just sufficient to meet the infant's metabolic requirements, without permitting accumulation of stores of the vitamin (16). Thus, if maternal vitamin A status is poor, breast-fed infants are likely to be subclinically vitamin-A-deficient by 6 months of age.

Vitamin A occurs in milk as a retinyl ester (mainly retinyl palmitate), located in the lipid fraction. The majority of milk vitamin A is derived from retinol bound to retinol-binding protein in the serum (24), which is esterified in the mammary gland (25). A minor but variable portion of milk vitamin A is derived from retinyl esters present in serum lipoproteins (24); this mechanism of vitamin A secretion into milk can be quantitatively important following a vitamin-A-rich meal (26).

Variations in breast-milk vitamin A content

To understand how the level of vitamin A in breast milk can be used as an indicator of vitamin A status, it is necessary to comprehend the nature of the relationship between milk vitamin A and vitamin A status, and the sampling factors that affect variations in measurements of milk vitamin A content. Neither animal studies (which often include a few groups with extreme levels of vitamin A status) nor population studies have adequately defined the relationship between milk vitamin A and maternal levels of the vitamin. The concentrations of vitamin A in milk and serum are similar, and significant correlations between these concentations have been reported in populations with relatively low vitamin A status (27, 28).[c] The relationship between milk vitamin A level and overall vitamin A status may resemble that of serum vitamin A and that in liver stores. However, whereas serum retinol concentration is tightly regulated by the liver when vitamin A stores are adequate, the concentration of vitamin A in milk may be less so. It has been postulated that newly absorbed dietary vitamin A can pass directly into milk (24), thus bypassing regulation by the liver.

The vitamin A status of the mother is not the only source of variation in the concentration of vitamin A in breast milk. Because milk vitamin A is located in the milk fat, the vitamin A concentration in a sample of milk is highly dependent upon the fat content of the sample. Unfortunately, the fat content of human milk is particularly liable to sampling errors (26) and this source of variation causes difficulties when breast-milk vitamin A is used as an indicator of mothers' and infants' vitamin A status.

The most important source of sampling variation is related to the fullness of the breast from which the milk sample is taken; the fuller the breast, the lower the fat content. A sample of milk obtained from a breast that the infant has not suckled for several hours will therefore have a relatively low fat level and hence a low level of vitamin A. Conversely, if a sample is taken from a breast recently used to feed the infant, a high fat level and a high vitamin A level will be obtained. Hence, if a large sample is taken, the first milk to be expressed will have a lower fat level than the last.

A second source of variation in milk fat (and thus in milk vitamin A level) is the time of day when the sample is taken. Generally the highest fat con-

centrations occur mid-morning, although the pattern of variation throughout the day is not entirely consistent (30). The variation throughout the day may be partly due to breast-feeding pattern, which would determine when the breasts are fuller or emptier.

Breast-milk vitamin A as an indicator: theoretical considerations

Target populations

In view of these biological characteristics of breast-milk vitamin A, what is its usefulness as an indicator of vitamin A deficiency at the individual and community levels? Theoretically, breast-milk vitamin A level could be used as an indicator of vitamin A deficiency in three target populations: women, breast-fed infants, and young children (1–3 years of age). For women, milk vitamin A level is an indicator of vitamin A status by virtue of its relationship to liver stores. Like serum retinol, the relationship of milk vitamin A concentration to liver stores is stronger when liver stores are low. Thus, milk vitamin A concentration is not a good indicator of the vitamin A status of individuals whose liver stores are in the adequate range, but could be a useful indicator for individuals or populations whose liver stores are low.

For predominantly breast-fed infants, breast-milk vitamin A concentration is an assessment of dietary intake of vitamin A. For all other segments of the population, quantitatively measuring dietary intake of vitamin A can be challenging. However, in most developing countries, complementary infant foods are typically low in vitamin A (31), and make a minor contribution to total intake; thus, assessing the vitamin A intake of breast-fed infants equates to understanding one food: mother's milk. Furthermore, because the vitamin A in breast milk is highly bioavailable, the link between intake of breast milk and infant vitamin A status is relatively strong. As an indicator of the vitamin A status of infants, the vitamin A concentration of breast milk would be expected to have a roughly linear relationship to infant stores of vitamin A.

Use of breast-milk vitamin A as an indicator of the vitamin A status of young children beyond infancy may be plausible, but insufficient data are available to evaluate this. In areas where weaning foods are low in vitamin A (i.e., most areas where vitamin A deficiency is a public health problem), breast milk levels of vitamin A may continue to be the most important predictor of vitamin A status into the sec-

[c] **Stoltzfus RJ.** *Vitamin A and lactation in Indonesian women.* Doctoral thesis, Cornell University, Ithaca, NY, 1992: 77–103.

ond or even third year of a child's life. For example, in rural Bangladesh, breast milk has been estimated to be the most important source of vitamin A in the diet of children up to the age of 27 months during most seasons of the year (31). Furthermore, to the extent that dietary vitamin A adequacy is similar among different subgroups of the population, there will be an association between women's and children's vitamin A status. If there is prior evidence to this effect, women's breast-milk vitamin A levels could be used as a proxy or sentinel measure of the vitamin A status of communities in general.

Assessment of individuals

For any particular target group, breast-milk vitamin A could be used to assess either individuals or populations; however, the methods used would differ. For assessment at the individual level, it is critical that the milk vitamin A content is that of a typical sample of milk secreted by a given mother. Thus, the sampling variability of milk fat discussed previously becomes paramount. This problem can be combated in one of the following ways: by collecting a milk sample that represents the typical milk fat secretion of a given mother; or by controlling for the milk fat concentration in the sample when the vitamin A content is analysed. The former method requires more sophisticated field protocols and compliance from the mothers, while the latter requires use of an additional laboratory method. The technical considerations of both strategies are discussed below.

For assessment at the population level, the samples collected from many mothers should be a fair representation of the milk consumed by infants in the community concerned; it is not essential that each milk sample represent the typical secretion of a given mother. For this purpose, casual samples of milk collected at random from women in the community are sufficient; the objective is to obtain a distribution of vitamin A levels in milk that can be used to describe the breast-milk vitamin A concentration in the community. Thus it is important to avoid any systematic bias in the way the samples are collected; for example, they should be collected at various times of day and at various intervals since the infant was last breast-fed.

Breast-milk vitamin A as an indicator: empirical evidence

Relationship to vitamin A status of women

Several studies have reported an association between serum and milk vitamin A concentration (27, 28).[d]

This association is illustrated by data from Indonesian women at 6 months' postpartum (Fig. 1). Milk vitamin A concentration was also associated with conjunctival impression cytology in these women (32). These associations support the relationship between milk vitamin A level and women's vitamin A status, but are not an adequate evaluation of milk vitamin A as an indicator of total levels, since serum retinol and conjunctival impression cytology are themselves imperfect indicators.[e] The best evidence that breast-milk vitamin A levels indicate women's vitamin A status is that these levels rise when vitamin A status is improved by supplementation. This causal relationship has been observed following supplementation with a single high dose of vitamin A (33, 34) and with continuous low-dose supplementation via use of vitamin-A-fortified foods (35, 36).

Relationship to vitamin A status of infants

The relationship between milk vitamin A concentration and infant vitamin A status has not been widely investigated. In Central Java, we observed a strong relationship (33). Infants whose mothers had breast-milk vitamin A concentrations >1.4 µmol/l had higher serum retinol concentrations than those infants whose mothers had breast-milk vitamin A concentrations below this cut-off value (Table 1). Furthermore, fewer infants whose mothers' milk contained at least 1.4 µmol/l vitamin A had evidence of depleted liver stores (as assessed by the relative dose–response test) compared with those whose mothers' milk had less vitamin A.

Use of breast-milk vitamin A to monitor and evaluate the impact of interventions

The most detailed report of the use of breast-milk vitamin A in programme evaluation is that of Arroyave et al., who used this approach to evaluate the national programme to fortify sugar with vitamin A in Guatemala (29). Breast-milk vitamin A concentrations along with several other indicators of the community's vitamin A status were measured before the fortification programme was implemented and subsequently at 6-month intervals over the first 2 years of the programme. Breast-milk vitamin A was evaluated in order to determine the extent to which the programme would improve the vitamin A intake of breast-fed babies. Since Guatemalan infants are fed only small amounts of complementary foods for as long as 1.5 years after birth, the major impact on infants from the sugar fortification programme was through an increase in vitamin A from breast milk.

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[d] See footnote c, p. 705.

[e] See footnote b, p. 704.

Fig. 1. **Association between breast-milk vitamin A and serum retinol concentration for Indonesian women at 6 months' postpartum.**
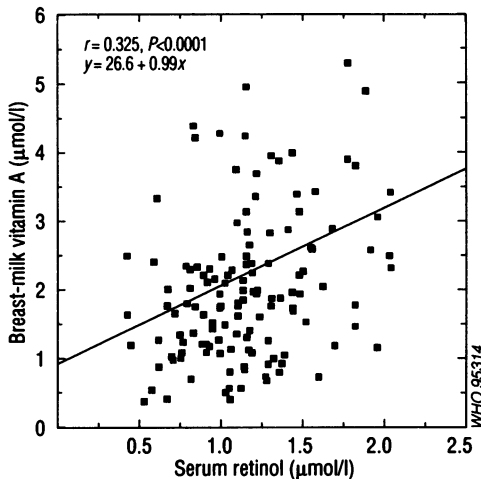
Breast-milk samples were collected by the dietary field workers, who were high-school graduates. The lactating mothers were asked to express manually about 8 ml milk from a "full" breast. This procedure proved to be feasible, easy, and well accepted by the mothers (29).

During the period of the fortification programme, the distribution of breast-milk vitamin A concentrations shifted significantly towards higher values. The breast-milk vitamin A content steadily increased during the first 18 months of the programme, then shifted slightly back towards lower values at the final evaluation round. The proportion of breast-milk vitamin A values <0.70 μmol/l fell from over 40% at baseline to a low of 12% after 18 months of the programme. The median milk concentration increased from 0.88 μmol/l at baseline to a peak of 1.40 μmol.

Table 1: **Association between low vitamin A concentration in mother's milk and infant's vitamin A status[a]**

Mother's milk vitamin A concentration (μmol/l)	Infant's serum retinol concentration (μmol/l)	% of infants with low vitamin A stores[b]
<1.4 (n = 42)	0.58 ± 0.21[c]	26[d]
≥1.4 (n = 83)	0.68 ± 0.23	13

[a] Data are for Indonesian mothers and infants at 6 months' postpartum. See ref. (33).
[b] Defined by abnormal relative dose–response test. See ref. (33).
[c] Mean ± SD; the means were significantly different by Student's t test, P = 0.010.
[d] Difference in proportions by χ^2 test, P <0.073.

Breast-milk vitamin A level has also been used to evaluate a programme to fortify monosodium glutamate (MSG) with vitamin A in Indonesia (36). For this purpose, breast-milk vitamin A was measured in control and programme communities 5 months and 11 months after introduction of fortified MSG in the programme areas. In the study, breast-milk samples (≥25 ml) were collected in the middle of a feed between 09:00 and 12:00 using a manual pump. The type of field worker responsible for collecting the samples was not described.

Only mean values for breast-milk vitamin A concentration were reported, ranging from 0.60 μmol/l at baseline to 0.67 μmol/l in the programme areas (a statistically significant increase). In the control areas, breast-milk vitamin A concentrations were similar to those in the programme areas at baseline, but subsequently declined slightly.

In another Indonesian study, breast-milk vitamin A concentration was used to measure the impact of high-dose supplementation of mothers in the postpartum period (33). The breast-milk vitamin A levels of supplemented mothers were 1.18 μmol/l higher than those of unsupplemented mothers at the first month postpartum. The breast-milk vitamin A concentrations of supplemented mothers remained significantly higher for at least 8 months' postpartum, whereupon the difference between supplemented and unsupplemented mothers was 0.48 μmol/l.

Advantages and disadvantages of breast-milk vitamin A as an indicator

In view of the variety of indicators of vitamin A status now available,[f] is there any advantage of using breast-milk vitamin A level rather than other indicators? Indicators of vitamin A status can be grouped into those that suggest clinical deficiency, subclinical deficiency, or dietary vitamin A adequacy. Breast-milk vitamin A concentration falls into the second of these categories, along with serum retinol concentration and relative dose–response methods for assessing vitamin A stores.[f]

One advantage of using breast-milk vitamin A rather than another indicator of subclinical deficiency is that collection of breast-milk samples is generally easier and more acceptable to survey participants. In our experience, this applies in cultures as diverse as those in Pakistan, Indonesia, Bangladesh, and Malawi. Milk can be collected by field workers with no clinical training; and local village women can become proficient at collecting milk samples.

[f] See footnote b, p. 704.

Mothers are very interested in the quality of their breast milk, which they can comprehend more readily than their serum retinol concentration. This common interest on the part of mothers, health planners, field workers and researchers may be advantageous in gaining community participation in vitamin A surveys or interventions where breast-milk vitamin A level will be assessed.

For programme evaluation purposes, breast-milk vitamin A level is a more responsive indicator than women's serum retinol concentration. For measuring the impact of high-dose vitamin A supplementation on mildly deficient Indonesian women, use of breast-milk vitamin A rather than serum retinol reduced the necessary sample size by half (37), thus lowering the costs of programme evaluation.

There are several restrictions that apply when breast-milk vitamin A is used. Female field workers are required in probably all cultural settings. Also, in milk the vitamin A, which is not protein-bound, is less stable than serum retinol, which is carried by retinol-binding protein. Thus, care must be taken to keep milk samples cold and protected from light during transport from the site of collection to the laboratory. These precautions, which are important also for serum retinol, are even more critical for milk vitamin A. Milk samples should be frozen at ≤-20 °C as soon as possible after collection. Furthermore, the laboratory procedure for measuring breast-milk vitamin A must be considered before a decision is made to use this indicator.

Practical aspects about determining breast-milk vitamin A to assess populations

Choosing the sample of women

Breast-milk vitamin A is most useful in assessing communities where breast-feeding is common and breast milk is the major infant food up to at least 6 months of age. In communities where breast-feeding is not the norm, lactating women may not be representative. Also, if breast milk makes only a minor contribution to the infants' diets, the breast-milk vitamin A concentration may not be a good indicator of infant vitamin A status.

Breast-milk samples should be collected from women who are producing mature milk and providing most of their infants' calories in this way. Milk samples collected in the first few weeks after birth will be higher in vitamin A, reflecting the transition from colostrum to mature milk. Thus, samples should be collected from women 1 month postpar-

tum and thereafter. If breast-feeding durations are long, milk samples collected from women at 8–12 months' postpartum can be included; however, if weaning occurs earlier, an earlier cut-off will provide a more representative sample.

The sample size required to monitor adequately the impact of an intervention depends on the size of the impact. From the Guatemalan and Indonesian fortification evaluations, information can be obtained about the required sample to measure low-dose interventions. Arroyave et al. reported the breast-milk data as the prevalence of vitamin A concentrations below a cut-off of 0.71 µmol/l (20 µg/dl) (29). Based on their data, we estimated that 350 samples were required to detect the improvement in breast-milk vitamin A at the first 6-month follow-up visit, 81 samples were required at 12 months, and 37 samples at 18 months.[9] The required sample size became smaller as the study proceeded because the impact of the intervention increased with time.

Mean values for breast-milk vitamin A are reported for the Indonesian MSG fortification study (36). From the data published in the study, we estimate that a sample size of 120 would be required to detect an improvement at either 5 months or 11 months after the intervention was begun.

Finally, using data from the postpartum supplementation trial of lactating Indonesian women, it has been calculated that 25 women would be sufficient to measure the impact at 2 weeks after the intervention began (the first month postpartum), while 60 women would be sufficient at 3 months' postpartum (37). In this case, a large impact was observed immediately, which subsequently decreased gradually.

Collecting milk samples

For assessment at the level of the individual, either a representative milk sample must be obtained or the vitamin A concentration has to be corrected for the fat content of the sample. A number of articles on milk sampling have appeared (38, 39), with the most appropriate method depending on what is being measured in the sample. The recommendations outlined below are specific for vitamin A.

• A good approximation of a representative sample is obtained by collecting all the milk from one breast that has not been used to feed the infant for a controlled length of time, e.g., 2 hours. Even better is to collect several such samples at different times of the day and mix them, but this is rarely acceptable in field settings. In Indonesia, we collected all the milk

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[9] For these and subsequent sample-size calculations, we have used the equation $n = (Z_\alpha + Z_\beta)^2 \sigma^2/\delta^2$, where $\beta = 80\%$ and $\alpha = 5\%$.

from one breast that had not been used to feed the infant for at least 1 hour (*33*). Care must be taken to operationalize collecting "all the milk"; for example, a simple manual pump can be employed until no more milk can be expressed from the breast using either the pump or manual expression by the mother. In this way, 10–150 ml of milk can be collected.

• For individual-level assessment, the alternative to collecting a representative milk sample is to determine the milk vitamin A per gram of milk fat. If corrected for milk fat, milk vitamin A concentrations determined from casual samples are adequate; it is not necessary to control the interval since the last feed or to collect all the milk from one breast. Milk fat can be determined by a simple procedure, as described below.

• For population-level assessment, casual samples of milk are adequate. Milk vitamin A does not need to be expressed per gram of milk fat; however, doing so may facilitate comparisons with other studies. Samples of 5–10 ml can be collected using a simple pump or by having women manually express their milk.

• A variety of breast pumps are available that do not require batteries or electricity; in the USA such pumps cost US$ 10–20. Pumps should be thoroughly cleaned between each use, but do not have to be sterilized.

• Because the vitamin A is present in the milk fat, it is important that the milk be homogeneous when aliquots are taken for determination of vitamin A and fat. Ideally, pre-measured aliquots of milk for vitamin A assay (and milk fat assay, if this is to be determined) should be taken just after the milk has been collected. Freshly expressed milk is warm and easily homogenized by gentle swirling. If it is not possible to divide the milk into aliquots at the time of collection, the sample should be quickly transferred to an opaque container and stored in an ice-filled vacuum flask for transport to the laboratory. The milk will separate into aqueous and cream layers during transport, but should be re-homogenized (by warming the sample to room temperature and swirling it gently for several seconds) and aliquoted before the samples are frozen. It will be apparent visually when the solid cream globules soften and emulsify and no solid cream remains stuck to the side of the container. The aliquots of milk should be stored at –20 °C.

Determining the vitamin A concentration of breast milk

Vitamin A in breast milk (and in serum) is commonly assayed using one of two methods: high-perfor-

mance liquid chromatography (HPLC) or spectrophotometry, with HPLC being more precise. Several HPLC methods are available that use some variation of the following basic steps (*40, 41*). Milk samples are saponified by incubation in a strong alkaline solution, which converts retinyl esters of vitamin A to retinol. The sample is then washed to remove the alkaline solution, and the vitamin A is extracted into an organic solvent, e.g., hexane or ether. A derivative of vitamin A is usually added to serve as an internal standard. The organic solvent is evaporated and the residue is redissolved in an alcohol. The sample is then assayed using reverse-phase HPLC with typically a water/methanol eluent. The advantages of this method are its accuracy and precision; the disadvantages are the cost of the HPLC equipment (US$ 15 000–30 000) and the technical expertise required to develop the assay and to maintain the equipment. In the hands of a good technician, this assay will run smoothly if the equipment is functioning well; when problems arise, troubleshooting and repairing the equipment can be challenging, especially in a developing country.

Alternatively, a spectrophotometric method can be used to determine the vitamin A in breast milk (*42, 43*). The sample is first saponified and the vitamin A and carotenoids are extracted into hexane or a mixture of xylene and kerosene. The absorbance of the sample extract is read at $\lambda = 460$ nm to determine total carotenoids, and at $\lambda = 328$ nm for retinol. The sample extract is then irradiated with UV light to photodecompose the retinol and the absorbances at $\lambda = 460$ nm and $\lambda = 328$ nm are re-read to determine the extent of destruction of retinol. The concentration of carotenoids and vitamin A are then calculated using an absorbance factor related to their respective extinction coefficients in the solvent mixture. The advantage of this method is that both the instrumentation and reagents are less costly than HPLC analysis. However, a high quality UV spectrophotometer is required and careful calibration is, of course, necessary.

Whichever method is used, rigorous quality assurance measures must be implemented in the laboratory. These include documentation of sample handling and storage, and routine analysis of external standards (e.g., retinyl palmitate) and of standard breast-milk samples. Standard specimens can be created by collecting a pool of milk and freezing standard aliquots to be run daily. The values obtained for the standards should be clearly documented in a laboratory notebook. These data should be monitored daily by the technician in charge of the assay and at least weekly by a supervisor. Deviations in the quality control sample that amount to more than two standard deviations of the normal values

require that the cause be identified and rectified and that all specimens analysed during the period concerned be repeated.

Determining milk fat concentration

The simplest procedure for determining milk fat is the creamatocrit method (44). For this purpose regular haematocrit tubes are filled with fresh milk samples and centrifuged. The cream layer rises to the top of the tube and is distinctly visible. The fat concentration is determined as a volume percent by dividing the length of the cream layer in millimetres by the length of the total specimen in the haematocrit tube; this is best accomplished using callipers.

Milk fat concentration is usually expressed as g/l; however, to correct for milk vitamin A concentrations, the volume percent determined by creamatocrit is sufficient. The vitamin A concentration is simply expressed as a ratio to the fat concentration in percent, e.g., 1.5 μmol/l vitamin A ÷ 5% fat = 0.3 μmol/% fat.

Presenting and analysing breast-milk vitamin A data

Breast-milk vitamin A data should be analysed in various ways. For comparisons of treatment or programme groups, both the mean values and the prevalence of low values in programme and control areas (or pre- and post-intervention values) should be compared. In presenting the prevalence of low breast-milk vitamin A concentrations, cut-offs of 0.7 μmol/l and 1.0 μmol/l have been used (29, 33); and the latter cut-off has recently been recommended by a WHO Expert Group.[h] Breast-milk vitamin A concentrations tend to be skewed towards high values; therefore, it is useful to report the median value in addition to the mean value, since the latter will be artificially high. Also, it may be useful to transform the data by taking the logarithm before comparing the means of groups.

Résumé

La teneur en vitamine A du lait maternel comme indicateur des besoins en vitamine A des femmes et des nourrissons

La teneur en vitamine A du lait maternel est un indicateur très utile pour évaluer les besoins en vitamine A des femmes allaitantes et des enfants nourris au sein. Cet indicateur est une méthode moins invasive que d'autres en ce qui concerne la mère et non invasive pour l'enfant. Il est généralement possible et bien accepté de recueillir des échantillons de lait.

La teneur du lait maternel en vitamine A semble être un indicateur particulièrement fiable pour mesurer l'impact des interventions comme les programmes d'enrichissement des aliments ou de distribution de suppléments de vitamine A pour les femmes. A cet égard, c'est un indicateur plus fiable que la concentration de rétinol sérique chez la femme.

Alors que l'on progresse sur la voie de l'éradication mondiale de l'avitaminose A, l'on recherche des méthodes plus efficaces pour déterminer les besoins en vitamine A et évaluer l'impact des programmes dans tous les groupes visés. La concentration de vitamine A dans le lait maternel semble être un indicateur utile à cet égard. De nouvelles études sur la teneur du lait maternel en vitamine A en tant qu'indicateur permettront d'en préciser et d'en affiner l'utilisation.

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[h] See footnote *b*, p. 704.

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